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Master in Biodiversity and Conservation Biology

Master Thesis

Amphibian road-kills in Mediterranean habitats

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Introduction

Circulation of motorized vehicles along road networks can affect populations, species, communities and ecosystems (Forman and Alexander, 1998; Trombulak and Frissell, 2000; Coffin, 2007). Road-kill mortality is the most well-known type of traffic impact (Forman and Alexander, 1998; Coffin, 2007). Some characteristics of the road and the surrounding area may affect road-kill risk (Forman et al., 2003). For example the proximity to habitat cover, to natural movement corridors or to artificial wildlife passages may increase road-kill rates (Hubbard et al., 2000; Clevenger et al., 2003; Forman et al., 2003). Traffic mortality may be also increased due to other factors like road (or road-network) location, when a way crosses an animal territory (Florida puma *Puma concolor coryi*: Kautz et al., 2006), distribution area (Iberian lynx *Lynx pardinus*: Ferreras et al., 1992) or migratory rute (Amphibians: Langton, 1989). On the other hand certain *taxa* show special vulnerability to road-kill mortality, for example the Amphibians, due to their low velocity of movement (increasing the time exposed to a risk), their inability to promptly detect the danger from a car and their escape inefficiency (their tendency to become immobilized when facing a threat; Scoccianti, 2001). Finally, several *taxa* are attracted by roads, increasing their mortality risk (see for example: Hodson, 1962; Fraser and Reardon, 1980; Antworth et al., 2005). Some of the most common attraction causes are basking and thermorregulating behaviors, described for Insects and Reptiles (Forman et al., 2003), and probably existing for some Amphibians too.

Amphibians are between the most threatened animal Classes (IUCN et al., 2008). They suffer from multiple threats related with global change, like land use modification, pollution and UV-radiation, the latter also affecting the infection by emerging diseases (Blaustein and

Wake 1990; Houlahan et al. 2000; IUCN et al., 2008). Such threats are leading Amphibians to a worldwide decline (Blaustein and Wake 1990; Houlahan et al. 2000; IUCN et al., 2008). Furthemore, Amphibians are the most impacted group of Vertebrates from traffic mortality (Glista et al., 2008), due to their already mentioned road-kill vulnerability and attraction by road surface (together with other factors like way characteristics and location). For this reasons in the last years traffic mortality is being considered as an additional factor contributing to the Amphibian global decline (Puky, 2006; Glista et al., 2008). In some cases road-kill rate may be high enough to exceed natural causes of mortality (like diseases and predation; Forman et al., 2003). In contrast to natural predation, traffic mortality is non-compensatory, and the kill rate is independent from density (Puky, 2006). Consequently traffic removes a constant proportion of a population potentially affecting rare species more heavily (Puky, 2006).

The Mediterranean basin is a Biodiversity hotspot, which holds at least 62 Amphibian species, including 32 endemism (Myers et al., 2000). Nowadays, about 25% of those Amphibian species are threatened (Cox et al., 2006), probably because in Mediterranean ecosystems all typical threats for Amphibians are widely represented. In such region UV-radiations may seriously affect Amphibian early stages (Macías et al., 2007). Furthermore, here land use changes, pollution and road network development quite increased in the last centuries (Underwood et al., 2009). The typical Mediterranean landscape is formed by a mosaic of urban, agricultural and natural areas. Within this scenario, areas with high traffic volume (towns and cultivations) are adjacent to protected areas. As a result, traffic impact on animal communities in Mediterranean ecosystems might be higher than in other environments. Despite this, there is a lack of knowledge about the traffic impact on Amphibian species in Mediterranean landscapes.

The main purpose of this work is to describe such impact by (1) quantifying the relative kill rates of Amphibian in a typical Mediterranean area, (2). analysing Amphibian road-kill phenology, and (3) determining the physical, behavioral and ecological characteristics that increase road-kill risk in Mediterranean Amphibian species.

Materials and methods

Study area

Our study was carried out in Doñana Natural Area (Doñana hereafter, south-western Spain: 36º59’ N, 6º26’W; Fig.1). This is a collection of areas (more than 50000 ha) with different levels of protection. The climate is Mediterranean sub-humid with Atlantic influence, with mild winters and hot summers. Average annual rainfall is 543 mm, although with alternating periods of wet and dry years (Meteorological Station of Doñana Palace; data of Natural Processes Monitoring Team of Doñana Biological Station). There are three main ecosystems: Mediterranean scrubland, dunes and wetlands (Valverde, 1958; Castroviejo, 1993). Doñana is a typical Mediterranean landscape, with a mosaic of urban, rural and natural areas. Consequently in this area the road network is capillar, with different infrastructure types and traffic intensities. We selected four roads inside our study area, one regional road (A494 Matalascañas-Mazagón, 23 km) and three forest/agricultural roads (Abalario Lane, 5 km; A483-Villamanrique de la Condesa, 16 km; A483-Hinojos, 11 km). These ones were the only paved roads inside the previously existing Doñana Natural Park. The community of Amphibians in Doñana shows a rich species complex (three Urodelan and eight Anuran species; Díaz-Paniagua et al., 2005), with the presence of all typical Mediterranean *taxa* (Appendix 1).

Data collection

From March 2006 to February 2007 (July 2006 excluded) we surveyed the four roads twice a week (the first early in the morning and the second in the late afternoon). Every road was covered in the two directions. One observer (the same during all study period) performed the survey driving at 15 km per hour, searching for road-killed animals on the road surface and in the adjacent verge/ditch. We recollected, identified and removed all road-killed vertebrates found.

To analyse Amphibian road-kill phenology, we explored the relation between the number of road-kills and monthly average rainfall and temperatures, two main parameters affecting Amphibian activity (Díaz-Paniagua et al., 2005). Climatic data were obtained from Meteorological Station of Doñana Palace (data of Natural Processes Monitoring Team of Doñana Biological Station).

To determine physical, behavioral and ecological characteristics that relates to road-kill risk, for every species we explored the relation among number of road-kills and weight, presence of massive migratory behavior, use of aquatic habitats and abundance rank. The species weight was selected to account for the lower detectability of the casualties of the smaller species (Puky, 2006). The presence of massive migratory behavior was selected considering that it is a factor known to affect road-kill mortalities in temperate latitudes (Langton, 1989; Forman et al., 2003; Puky, 2006). The third variable, use of aquatic habitats, was selected considering that in temperate latitudes the more aquatic species seem to be less affected by road-kill risk (Blab, 1986). The abundance rank was selected considering that is a factor that heavily affects road-kill risk (Forman et al., 2003). Data concerning this variables were obtained from bibliography (Arnold and Ovenden, 2002; Díaz-Paniagua et al., 2005), and using information from our study area or nearby (Appendix 1).

Data analysis

We analysed the phenology by fitting generalized linear models (GLMs; McCullagh and Nelder, 1989), using the GENMOD procedure for SAS version 9.1 (SAS Institute, 2003). We fitted three models, the first with all the road-killed Amphibians, and the latter two only with the more prevalent species. We used negative binomial error distribution and log-link functions in all models. The dependent variable was the number of road-kills per month. The independent categorical variables were monthly average rainfall and temperatures.

We analysed the species characteristics by fitting generalized linear mixed models (GLMMs; McCullagh and Nelder, 1989), using the GLIMMIX procedure for SAS version 9.1 (SAS Institute, 2003). With the aim to determine which characteristics affect presence or absence of road-kills we fitted a first GLIMMIX with Binomial error distribution and logit-link function. The dependent variable was presence/absence of road-kills per sample for every species that could be potentially road-killed. We used the presence of massive migratory behavior, selection of aquatic habitats and the abundance as independent categorical variables, and the species weight as independent continous variable. Then, we fitted a second model with Poisson error distribution and log-link function to the number of casualties per sample for every road-killed species. We used presence of massive migratory behavior, selection of aquatic habitats and abundance as potential predictors. We used the date of every sample as a random variable to control for the effect of environmental variables such as temperature and precipitation.

Results

During the whole survey we recollected 2301 road-killed Vertebrates, whereof 1588 were Amphibians (Tab.1). Of these, the majority were Anurans (1544 individuals; Tab.1). Western spadefoot toad *Pelobates cultripes* (142 individuals), Natterjack toad *Bufo calamita* (381 individuals) and Iberian ribbed newt *Pleurodeles waltl* (20 individuals) were the species with more identified victims (Tab.1; Appendix 1).

Monthly average rainfall significantly affected the phenology of the total number of road-killed Amphibians ($F = 8.62$; $p = 0.0188$; Fig.2). In the same way, monthly average rainfall significantly affected Western spadefoot toad road-kill phenology ($F = 10.34$; $p = 0.0123$; Fig.3). Both precipitation ($F = 6.97$; $p = 0.0297$; Fig.4) and temperature ($F = 21.45$; $p = 0.0017$; Fig.4) affected Natterjack toad road-kill numbers. In all the three cases we detect an increase of casualties corresponding to higher precipitations. We may apreciate that the annual road-kill peak for the three cases perfectly correspond to the annual precipitation peak (during Octuber and November; Fig.2; Fig.3; Fig.4). Concerning the Natterjack toad, we may observe a significant road-kill decrease corresponding to the increase of temperatures (from May to September; Fig.4).

About species characteristics analysis, results from first GLIMMIX procedure show that species with massive migratory behavior significantly suffer road-kill risk ($F = 17.03$; $p < 0.0001$; Fig.5). From the same analysis we may observe that species that mainly select aquatic habitats were less detected in road-kill surveys ($F = 31.14$; $p < 0.0001$; Fig.6). Furthermore, species that show higher abundance rank were significantly affected by road-kill probability ($F = 27.57$; $p < 0.0001$; Fig.7). Finally the weight had not effects ($F = 0.43$; $p = 0.5123$).

Results from second GLIMMIX procedure show that road-kill magnitude decrease in species with massive migratory behavior ($F = 14.55$; $p = 0.0005$; Fig.8) and in species that actively select aquatic habitats too (F = 18.50; p = 0.0001; Fig.9). Species abundance rank had not effects (F = 1.66; p = 0.2053).

Discussion

Amphibians were the most common group of road-killed vertebrates in our Mediterranean site, as already described in temperate latitudes (Puky, 2006; Glista et al., 2008). The conservation status of this group is alarming across the Mediterranean basin (Cox et al., 2006), hence we should concentrate our efforts to mitigate road mortality by using measures nowadays applied in temperate latitudes (for example the use of barriers that route Amphibians to artificial passages; Forman et al., 2003; Puky, 2006). Among mainly affected species, the Western spadefoot toad and the Iberian ribbed newt are characterized by their relatively small distribution (mostly Iberian Peninsula) and are listed as near threatened in Spain (Montori et al., 2002; Tejedo and Reques, 2002), showing negative population trends in our study area (Díaz-Paniagua et al., 2005). Conservation efforts are necessary to preserve Doñana populations and the entire species from traffic threat.

The influence of rainfall (more road-kills associated to more precipitations) is linked to the typical Mediterranean climate of our study area: the extended summer drought forces Amphibians to concentrate their reproduction during wet periods, when precipitations provide water at permanent and temporal ponds (Díaz-Paniagua et al., 2005). Consequently, Amphibian terrestrial activity overlaps with rainfall peaks (mainly Autumn), due to their necessity to reach the breeding areas (Díaz-Paniagua et al., 2005). We did not find relation between monthly average temperatures and road-kill phenology, despite the existence of bibliography suggesting that several of the species present in Doñana have very specific temperature requirements to start their activity and reproduction (Díaz-Paniagua et al., 2005).

Probably such species are not enough to influence the entire analysis, due to the presence of eurythermal species in Doñana.

We found for Western spadefoot toad the same results that for the entire Class *Amphibia*. For the reproduction this species needs temporal ponds with wide open surfaces, that only appear after intense precipitations (Díaz-Paniagua et al., 2005). For such reason the mortality of this toad (and presumably its activity) perfectly matches rainfall patterns. Instead, road-kill of Natterjack toad seems to be also affected by the monthly average temperature. This species breeds in small temporal ponds and flooded environments, which persistence mainly depend on such climatic factor (Díaz-Paniagua et al., 2005). Obviously, in the case of Natterjack toad too the precipitations continue to be a determinant factor for the formation of breeding habitats, the activity increase an the consequent road-kill casualties.

In regard with the species characteristics, the lack of weight effect despite the lower detectability of of the smaller species (Puky, 2006) shows that we were able to detect them in our survey. As previously described at temperate latitudes (Langton, 1989; Forman et al., 2003; Puky, 2006), in Mediterranean habitats the massive migratory behavior involve high road-kill risk. Only two of the species present inDoñana show this behaviour: the Western spadefoot toad and the Iberian ribbed newt (Díaz-Paniagua et al., 2005). The latter, despite its low abundance in our survey area, results affected by relatively high road mortality. We also confirmed that in Mediterranean environments the extrictly aquatic species suffer lower road mortality, as described in central Europe (Blab, 1986). Aquatic species live in permanent ponds most of the year, and as an alternative they can live in temporal waters and remain inactive under ground (estivation) during the summer drought (Díaz-Paniagua et al., 2005). As an example, the Southern marbled newt *Triturus pygmaeus* and Perez's Frog *Pelophylax perezi*, both species quite abundant in the area (Díaz-Paniagua et al., 2005) are not seriously affected from road-kill risk. Finally, the abundance rank of the species affect road-kill risk too, as described for other Vertebrates (Forman and Alexander, 1998; Trombulak and Frissell, 2000; Forman et al., 2003).

The last analysis concerning road-kill magnitude showed that the species with massive migratory behavior were significantly less detected during road-kill surveys, contrary to the results of previous analyses. So the massive migratory behavior affect road-kill risk, but does not seem to influence road-kill magnitude. This probably is a statistical artefact in this second analysis, due to reduced data set in which main part of casualties correspond to a species (Natterjack toad: 16.56% of all road-killed Vertebrate) that not show massive migratory behavior. Concerning this second analysis too, selection of aquatic habitats continue to be a significant factor, with decrease of casualties in extrictly aquatic species. Finally, the abundance rank had not effects on road-kill magnitude. Probably this variable loss its importance in this second analysis due to the Iberian ribbed newt, that is relatively scarce in our survey area (Díaz-Paniagua et al., 2005) but due to its massive migratory behavior is in the third ranking of road-killed species.

Future purposes

In this work we did not consider the spatial distribution of the road-casualties and the potential factors related to it which can give an idea of where and how we can mitigate the impact of roads. Therefore we plan to analyse which are the main characteristics of the surveyed roads and their surrounding areas and how they relate to the presence and number of victims. We will also search for the relation among road-kill risk in Mediterranean habitats and some variables like traffic intensity, car speed, road topography, distance to wildlife artificial and natural passages, vegetation type and proximity to continental waters. On the other hand we will repeat all the analyses for every Vertebrate Class (Reptiles, Birds and

Mammals), with the addition of a global analyses for all Vertebrates together. The final purpose of this work will be describe and understand which factors determine road-kill in Mediterranean areas, for the elaboration of management measures focused to the conservation of Mediterranean biodiversity in the imperative twinning with human sustainable activities.

Aknowledgements

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Tab.1: Road-killed individuals for every detected *taxon*, and corresponding percentages. All the percentages refferred to the overall of road-kills.

***Taxa*:**	**Road-killed individuals:**	**Road-killed percentages:**
AMPHIBIA:	1588	69.01%
Unidentified Amphibian	14	0.61%
Caudata:	20	0.87%
Pleurodeles waltl	20	0.87%
Anura:	1554	67.10%
Unidentified Anuran	1023	44.46%
Bufo calamita	381	16.56%
Pelobates cultripes	142	6.17%
Pelophylax perezi	7	0.30%
Discoglossus galganoi	1	0.04%
REPTILIA:	304	13.21%
AVES:	218	9.47%
MAMMALIA:	191	8.30%
Total:	2301	100%

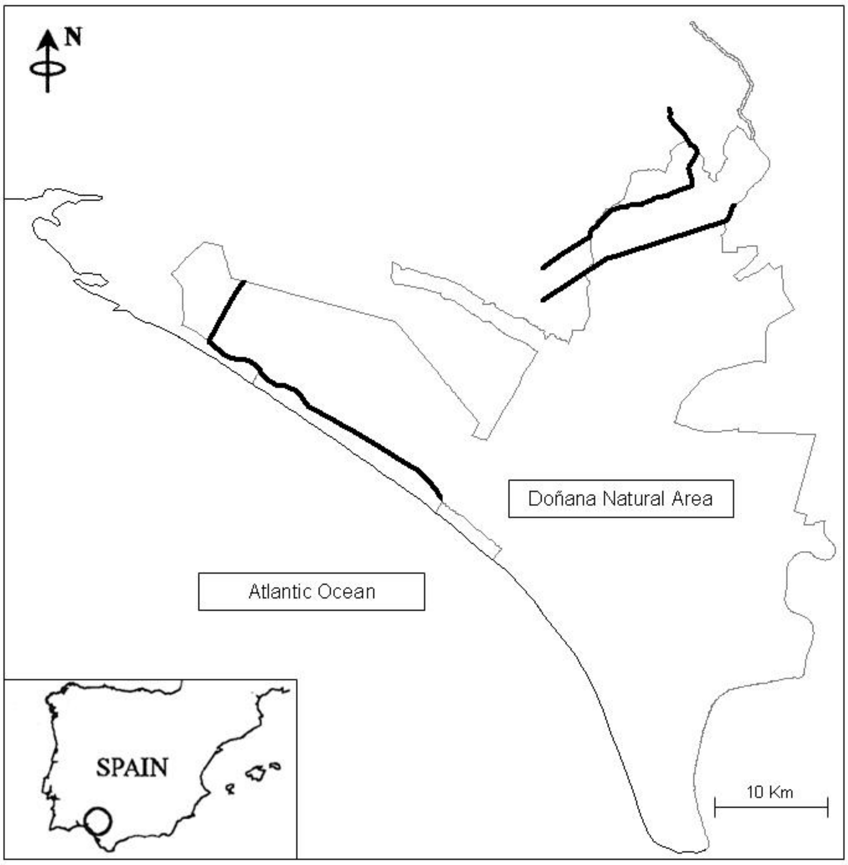

Fig.1: Study area (Doñana Natural Area). Black lines are the sampled roads.

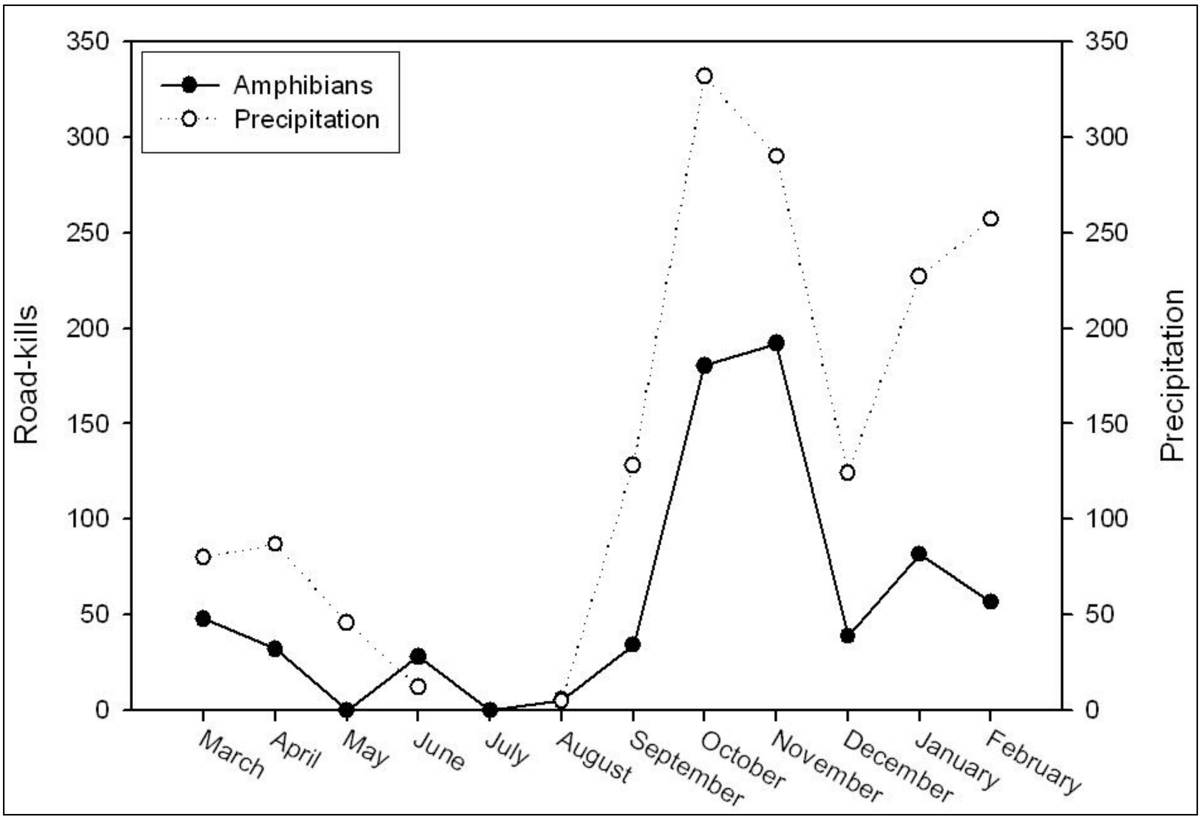

Fig.2: Road-killed Amphibians and monthly average rainfall (mm) along the whole survey.

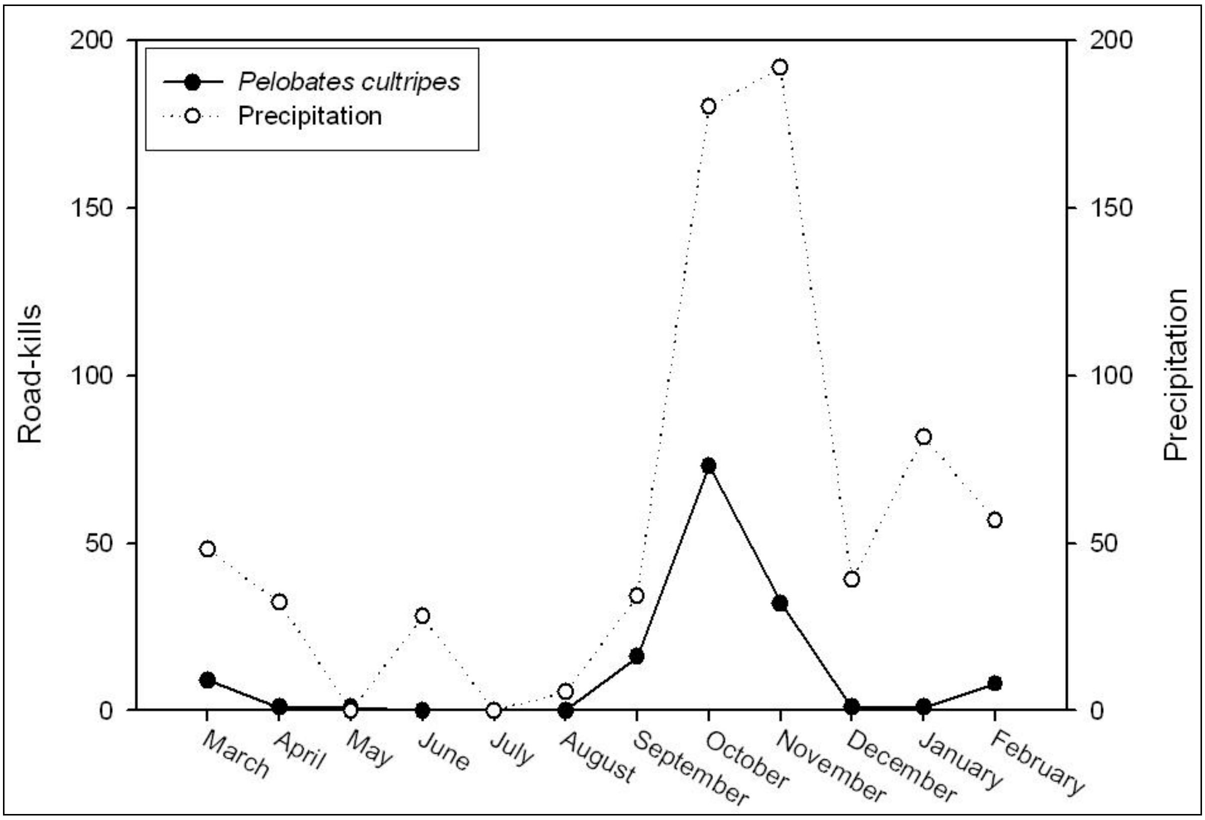

Fig.3: Road-killed Western spadefoot toads and monthly average rainfall (mm) along the whole survey.

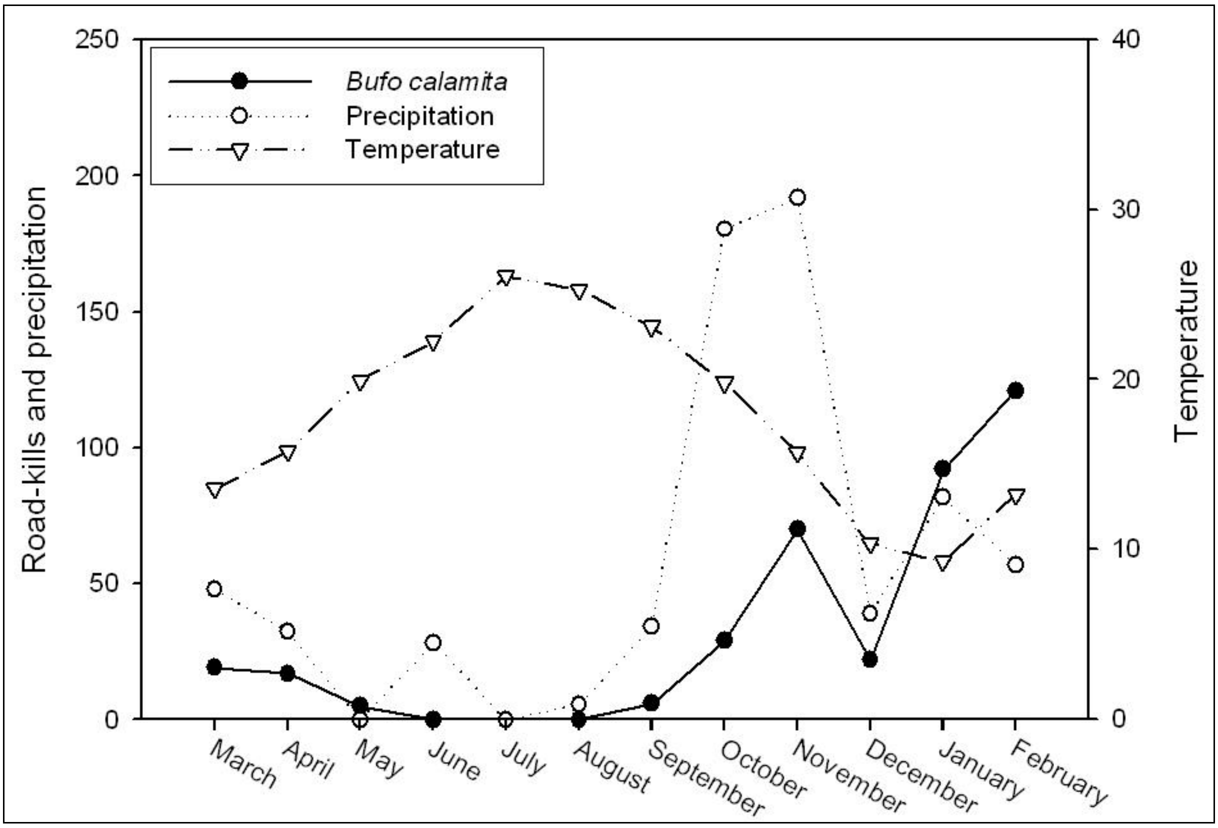

Fig.4: Road-killed Natterjack toads, monthly average rainfall (mm) and monthly average temperatures (ºC) along the whole survey.

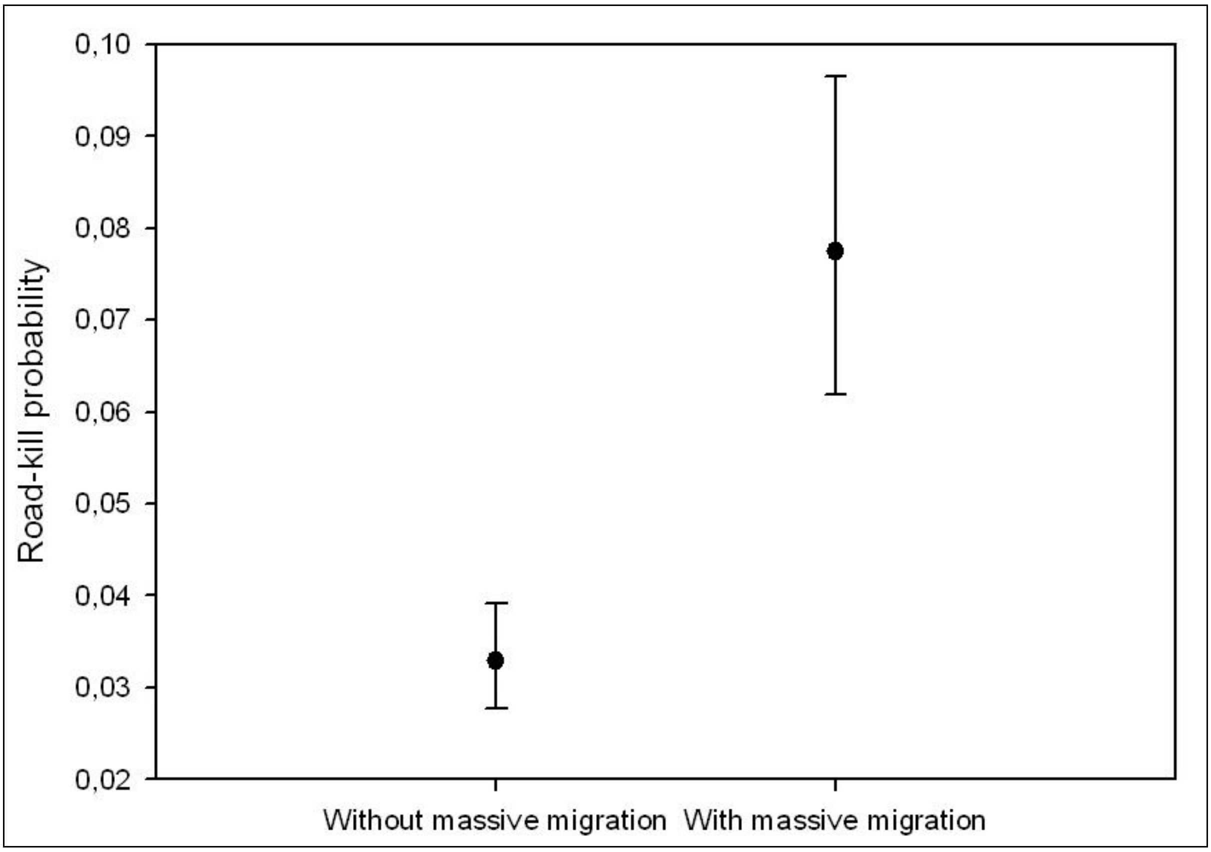

Fig.5: Road-killed probability for Amphibian species with and without massive migrations.

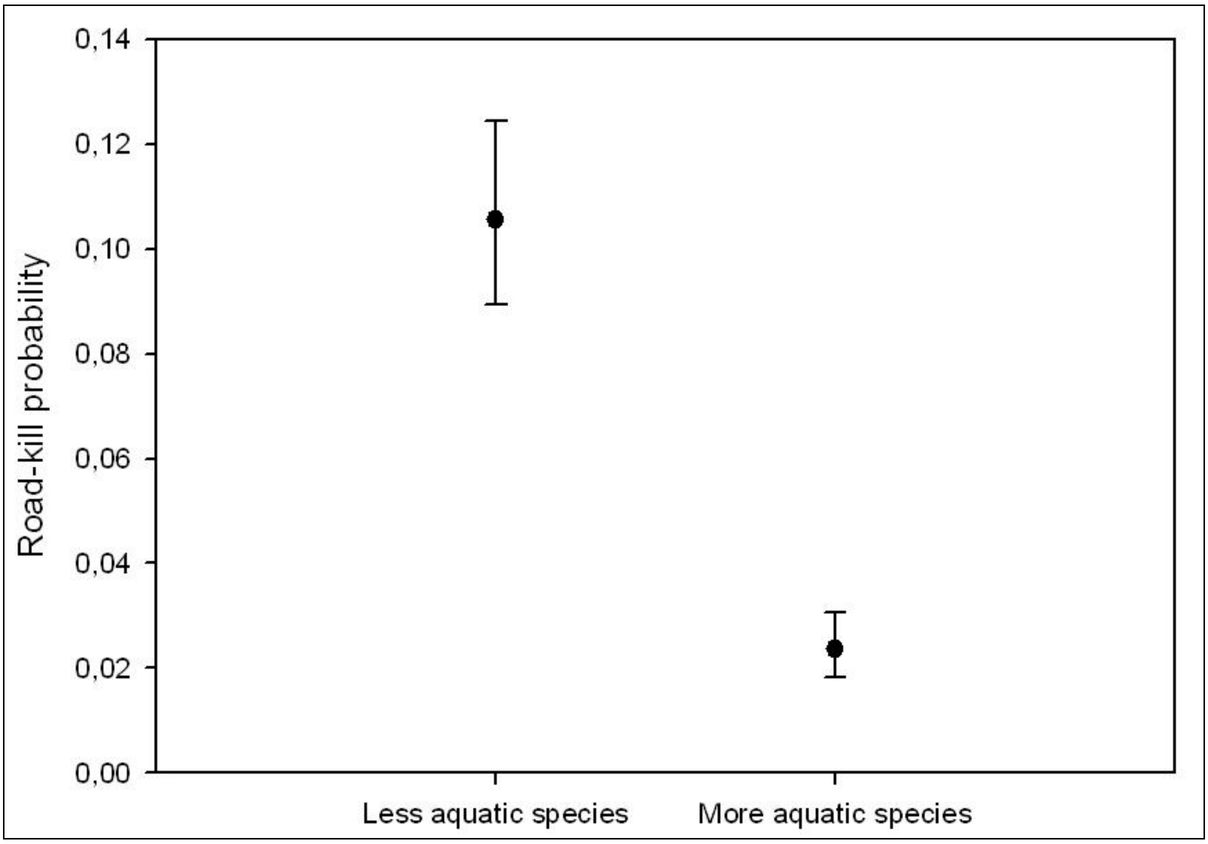

Fig.6: Road-killed probability for Amphibian species more or less related with aquatic habitats.

Fig.7: Road-killed probability for scarce and abundant Amphibian species.

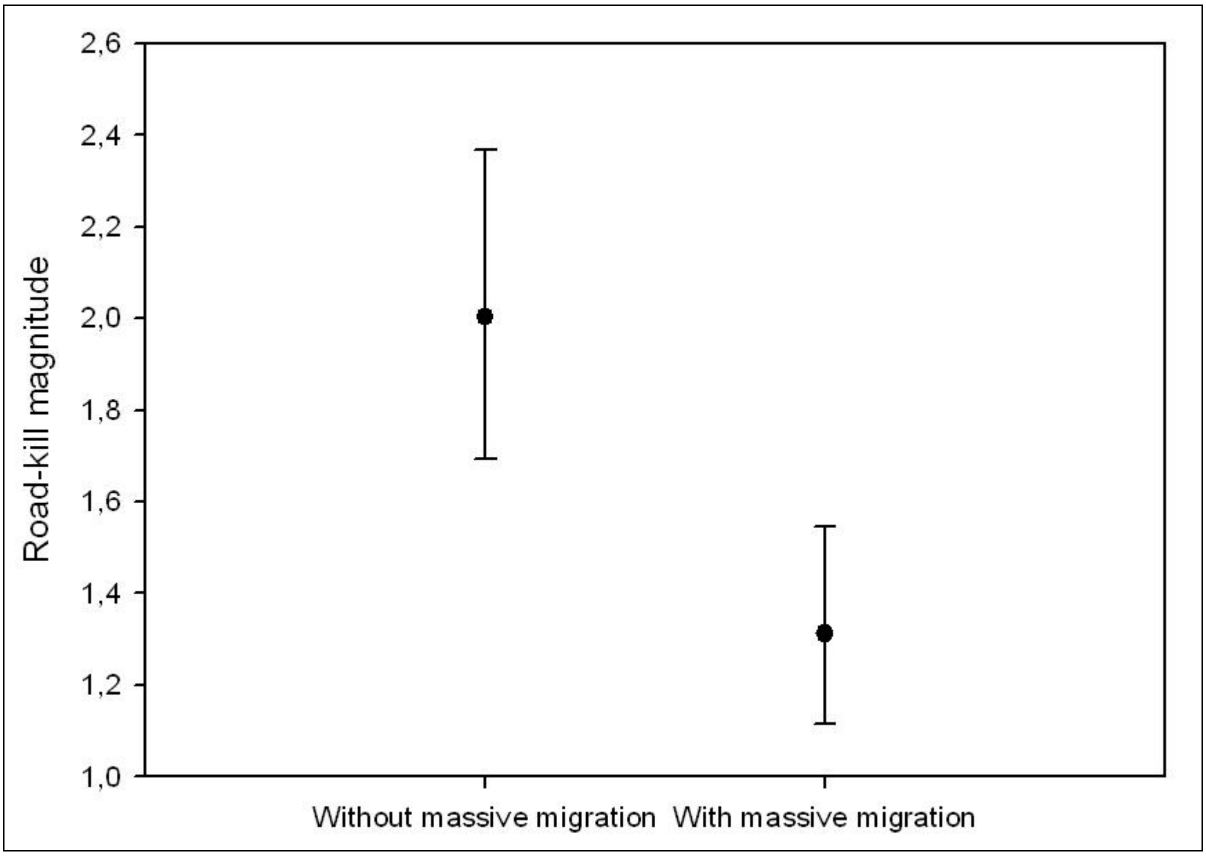

Fig.8: Road-kill magnitude in Amphibian species with and without massive migrations.

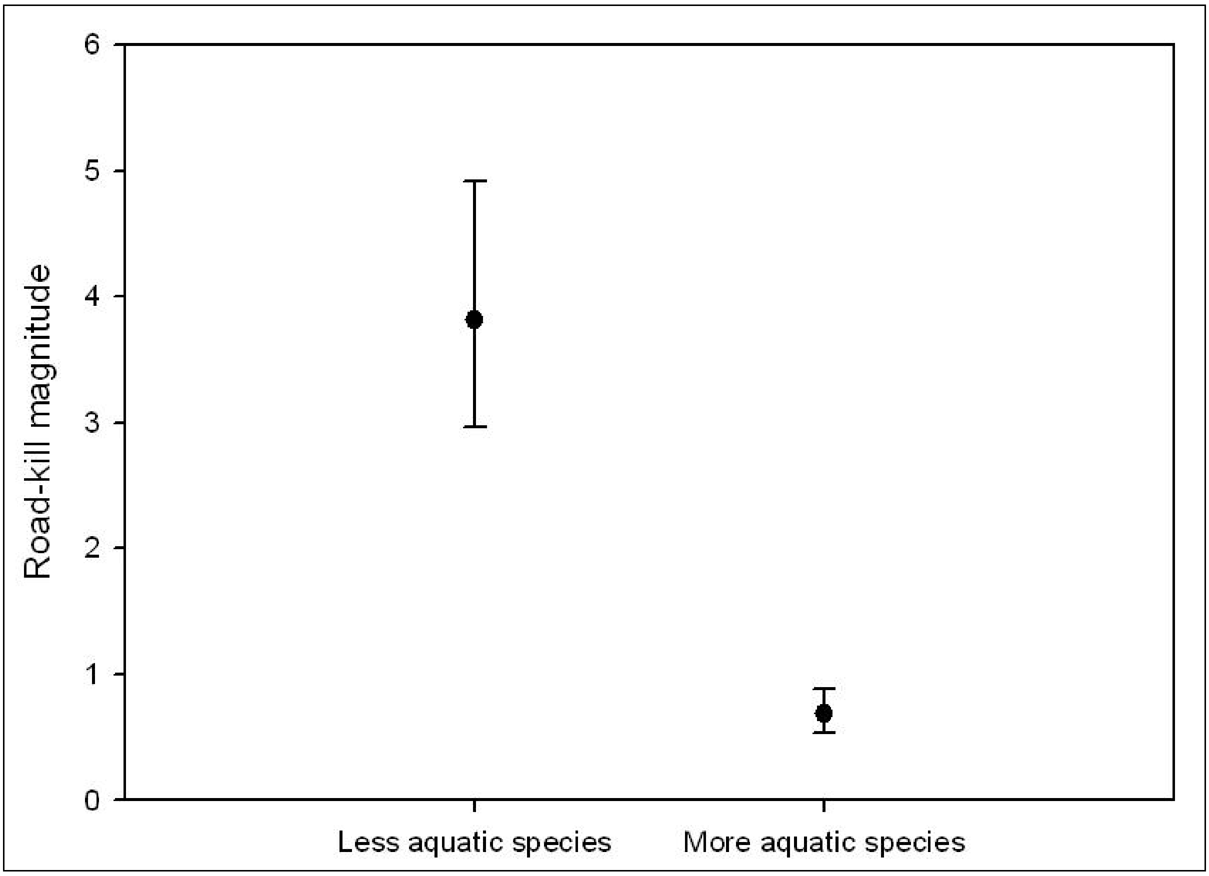

Fig.9: Road-kill magnitude for Amphibian species more or less related with aquatic habitats.

Appendix 1: physical, ethological and ecological measured characteristics for DNA Amphibian species. Weight is expressed in grams.

Species	Road-killed individuals	Weight	Migratory behavior	Aquatic	Abundance
Caudata:					
Iberian ribbed newt (*Pleurodeles waltl*)	20	25.200	1	1	0
Southern marbled newt (*Triturus pygmaeus*)	0	2.150	0	1	1
Bosca's newt (*Lissotriton boscai*)	0	0.525	0	1	0
Anura:					
Iberian midwife toad (*Alytes cisternasii*)	0	6.400	0	0	0
Iberian painted frog (*Discoglossus galganoi*)	1	9.300	0	1	0
Western spadefoot toad (*Pelobates cultripes*)	142	17.800	1	0	1
Iberian parsley frog (*Pelodytes ibericus*)	0	2.500	0	1	0
European toad (*Bufo bufo*)	0	149.300	0	0	0
Natterjack toad (*Bufo calamita*)	381	12.500	0	0	1
Mediterranean tree frog (*Hyla meridionalis*)	0	2.300	0	0	1
Perez's Frog (*Pelophylax perezi*)	7	15.400	0	1	1